

### ***Amendments to the Specification***

Please replace current page 1 with amended page 1 that is appended hereto.

Please replace paragraph [0021] with the following amended paragraph:

[0021] ~~Figure-6~~ Figures 6A-C: Unit assay for Taq DNA polymerase in the presence of SSB (AccuPrime protein or E. coli SSB) under various conditions. Unlike E. coli SSB which shows general tendency of inhibition as the protein concentration increases, AccuPrime protein enhances Taq DNA polymerase unit activity in a concentration-dependent manner where the optimal enhancement is achieved under a sub-optimal condition for the polymerase at AccuPrime protein concentration of 0.1 mg/50 ml reaction.

Please replace paragraph [0023] with the following amended paragraph:

[0023] ~~Figure-8~~ Figures 8A-D: Scan profile of alkaline agarose gel electrophoresis for primer extension products by Taq DNA polymerase using a specific primer and single stranded circular M13mp19 DNA as a template, in the presence or the absence of AccuPrime protein: (A) primer extension in the absence of AccuPrime protein; (B) with 50 ng AccuPrime protein/50 ml rxn; (C) with 100 ng AccuPrime protein/50 ml rxn; and, (D) with 100 ng MthSSB/50 ml rxn. Results show that in the presence of 100 ng of AccuPrime protein in 50 ml rxn, the peak population of extension products shifted toward lower molecular weight indicating the polymerase extending the primer shorter in the presence of AccuPrime protein than those in the control. This phenomenon was most obvious at 1.5 min time point. The second peak showing on top of the gel in the bottom panels (C and D) is the primer from the top panel.

Please replace paragraph [0025] with the following amended paragraph:

[0025] ~~Figure-10~~ Figures 10A & B: Real-time stability assay for AccuPrime Taq PCR Reaction Mixes and SuperMixes up to 6 month at room temperature. Each sample was duplicated: Panel A Lanes 1 and 12, Platinum Taq DNA polymerase control; Lane 2,

AccuPrime Taq PCR Reaction Mix I (RMI) control; Lanes 3-6, RMI after incubation at RT for 1, 2, 3 and 6 month, respectively; Lane 7, AccuPrime Taq PCR Reaction Mix I without glycerol (RMI-gly) control; Lanes 8-11 RMI-gly after incubation at RT for 1, 2, 3 and 6 month, respectively; Lane 13, AccuPrime Taq PCR SuperMix I (SMI) control; Lanes 8-11 SMI after incubation at RT for 1, 2, 3 and 6 month, respectively. Panel B shows counter parts of AccuPrime Taq PCR Reaction Mix II with and without glycerol, and AccuPrime Taq PCR SuperMix II as shown in Panel A.

Please replace paragraph [0026] with the following amended paragraph:

[0026] ~~Figure 11~~ Figures 11A & B: TOPO TA cloning with PCR amplification products from AccuPrime Taq DNA polymerase. The PCR amplification products using two different primer sets (FIG. 11A: SEQ ID NO: 158 and FIG. 11B: SEQ ID NO: 159) were cloned into pCR2.1 TOPO vector, transformed TOP10 cells and selected 6 transformants randomly from each transformation. Plasmids purified from the transformants were checked for the right insert and sequenced the flanking region to make sure they were flanked by TT at 5' end and AA at 3' end. The sequencing showed the right insert flanked by TT and AA (blue arrows) indicating AccuPrime Taq DNA polymerase adds 3' A overhang necessary for TOPO TA cloning.

Please replace paragraph [0029] with the following amended paragraph:

[0029] ~~Figure 14~~ Figures 14A & B: Performance comparison of AccuPrime Taq DNA polymerase with Hot Star Taq (Qiagen) using two sets of primers based on the size of the amplicons; (A)  $\beta$ -globin, 468 bp;  $\beta$ -globin, 731 bp; c-myc, 822 bp;  $\beta$ -globin, 1100 bp; and Hpfh, 1,350 bp, (B)  $\beta$ -globin, 2.2 kb; and  $\beta$ -globin, 3.6 kb. AccuPrime Taq performed consistently with a high specificity regardless of the size of the amplicon up to 3.6 kb, while Hot Start Taq were more prone to produce non specific bands as the amplicon size increased.

Please replace paragraph [0030] with the following amended paragraph:

[0030] ~~Fig. 15~~ Figures 15A & B: Discrimination against false priming site by AccuPrime Taq DNA polymerase, compared with Taq DNA polymerase or Hot Star Taq (Qiagen). A false priming site was introduced by 13 base homology in two different locations of the template, separated by 350 bp, where 13 nucleotides of the 3' end of the reverse primer could anneal to (SEQ ID NO: 160). The remaining 7 nucleotides of the 20 nucleotide long reverse primer anneals only to the genuine priming site (13951) (SEQ ID NO: 161). Only the AccuPrime Taq discriminated against the 13 base homology priming while maintaining a high yield.

Please replace paragraph [0033] with the following amended paragraph:

[0033] ~~Figure 17~~ Figures 17A & B: Feasibility assay for PCR miniaturization using AccuPrime Taq DNA polymerase. Unlike Taq DNA polymerase alone, AccuPrime Taq DNA polymerase functions efficiently regardless of the reaction volume and the amount of the enzyme itself could be lowered proportionally to the reaction volume without losing the robustness or specificity of the reaction.

Please replace paragraph [0046] with the following amended paragraph:

[0046] ~~Figure 30~~ Figures 30A & B: SDS polyacrylamide gel electrophoresis (Novex 4-20% Tris Glycine gel) for cross-column analysis of the fractions from EMD-SO<sub>3</sub> column from BL21(DE3) host. Lanes in the gel contain: M) markers; 1) lysate; 2) heat supernatant; 3) flow-through; 4) wash with 50 mM NaCl; 5) fraction #29 (2.5 ml fractions); 6) #31; 7) #33; 8) #35; 9) #36; 10) #38; 11) #40; 12) #42; 13) #44; 14) #46; 15) #48; 16) #50; 17) #52; 18) #54; 19) #56; 20) #60; 21) #65; and 22) #69. The gel shows that while AccuPrime protein II elutes in two peaks as before (Fig. 2), the second peak still contains a considerable amount of contaminants.

Please replace paragraph [0048] with the following amended paragraph:

[0048] ~~Figure 32~~ Figures 32A-C: Endonuclease activity assay using supercoiled circular plasmid ( $\phi$ X174) incubated with varying amounts of AccuPrime proteins in 50  $\mu$ l

reaction solution at 37°C for 1hr. The resulting plasmid was mixed with 5 µl of 10x BlueJuice and analyzed on 0.8% agarose gels for appearance of relaxed circular or linear DNA. Lanes 1 to 4 were from samples made from commercial Platinum Pfx Amplification buffer with 0, 0.75 (2.5x), 1.5 (5x) and 3 (10x) µg of AccuPrime Protein II (APP II), respectively. Lanes 5 to 8 were identical to lanes 1 to 4, except all the components were assembled for the pilot lot. Lanes 9 to 12 contain AccuPrime Protein I (APP I) at the amount of 0, 0.5 (5x), 1 (10x) and 2 (20x) µg, respectively. Panel (A) samples were in 1x BlueJuice and loaded to the gel without heating. Panel (B) samples were heated at 95°C for 5 min in 1x BlueJuice, and loaded on the gel. Panel (C) samples were heated at 95°C for 5 min in 1x BlueJuice and 0.5% SDS, and loaded on the gel. The gels clearly show strong binding of AccuPrime Protein II that resulted in shift in mobility of the DNA band and resistant to heat treatment without SDS. AccuPrime protein I came off from the DNA upon heating at 95°C for 5 min even without SDS.

Please replace paragraph [0057] with the following amended paragraph:

[0057] ~~Figure 41~~ Figures 41A-C: Use of *Methanococcus jannachii* SSB in cycle sequencing with ABI Prism® BigDye™ Terminator Cycle sequencing Kits (FIG. 41A: SEQ ID NO: 162; FIG. 41B: SEQ ID NO: 163; FIG. 41C: SEQ ID NO 164).

Please replace paragraph [0061] with the following amended paragraph:

[0061] ~~Figure 45~~ Figures 45A & B: (A) SDS gel of EMD-SO4 fractions. L is load, FT is load flow through. Fractions 26-30 were pooled. (B) Pooled fractions were dialyzed and 2 or 5 ug were run on SDS gel with the purified Sso SSB from Codon Plus cells. 1 is original from Codon Plus, 2 is rSso SSB from BL21 DE3.

Please replace paragraph [0115] with the following amended paragraph:

[0115] Compositions of the invention may be used, e.g., in “hot-start” nucleic acid synthesis, where a reaction is set up at a temperature such that anti-DNAP antibodies and/or anti-RT antibodies can ~~exhibit~~ inhibit nucleic acid synthesis and where nucleic

acid synthesis subsequently is initiated by increasing the temperature to reduce inhibition by the anti-DNAP antibodies and/or anti-RT antibodies. Thus, the invention provides a method for synthesizing a nucleic acid involving: (a) mixing one or more templates with one or more anti-DNAP antibodies and/or one or more anti-RT antibodies and/or one or more SSBs (or combinations thereof) to form a mixture; (b) incubating the mixture under conditions sufficient to inhibit or prevent nucleic acid synthesis; and (c) incubating the mixture under conditions sufficient to make one or more nucleic acid molecules complementary to all or a portion of said templates (*i.e.*, a primer extension product). Reaction conditions sufficient to allow nucleic acid synthesis (e.g., pH, temperature, ionic strength, and incubation time) can be optimized according to routine methods known to those skilled in the art and may involve the use of one or more primers, one or more nucleotides, one or more buffers or buffering salts, one or more RTs and/or one or more DNAPs (or combinations thereof).

Please replace paragraph [0186] with the following amended paragraph (please note that paragraph title has not been added, but was underlined in the original text):

[0186]        Exo-nuclease activity. 100 pmol of oligonucleotide (36mer; 5'-GGG AGA CGG GGA ATT CGT CGA CGC GTC AGG ACT CTA-3' (SEQ ID NO:1)) was labeled with <sup>32</sup>P at the 5' end using 10 units of T4 polynucleotide kinase and 10 μCi of [γ-<sup>32</sup>P] ATP in 50 μl of 1x PNK exchange buffer. The reaction mix was incubated at 37°C for 30 min and the reaction was terminated by incubating the mix at 70°C for 10 min. Unincorporated nucleotides were removed by eluting the reaction mix through Amersham-Pharmacia Micro Spin G-25 column twice following the manufacturers instruction.

Please replace paragraph [0188] with the following amended paragraph (please note that paragraph title has not been added, but was underlined in the original text):

[0188]        Single stranded DNA binding. AccuPrime protein affinity for secondary structure of the single stranded DNA was tested with the 84 mer synthetic

oligonucleotide KP\_PALIN\_cont: 5'-CTC CTG GAT CGA CTT CAG TCC GCT GAT GAT TAG ATG TCG TCC TGG ATC GAC TTC ACT CCG CAC CCG CTA CCA ACA ACA GTA CCC-3' (SEQ ID NO:2). The oligonucleotide was radiolabeled at the 5' end in the same manner as the 5' (ss) substrate for the exo-nuclease activity assay above with the oligonucleotide concentration at 5  $\mu$ M.

Please replace paragraph [0192] with the following amended paragraph:

[0192] For more defined mechanistic studies, pre-primed single stranded circular M13mp19 DNA was used in place of nicked salmon testes DNA as template. The primer, M13mp19\_1442L30, used in this study was designed to anneal to coordinate 1442 of the (+) strand of M13mp19 DNA and has the sequence: 5'-GCC GAC AAT GAC AAC AAC CAT CGC CCA CGC-3' (SEQ ID NO:3).

Please replace paragraph [0200] with the following amended paragraph:

[0200] After the period of incubation, the reaction mix (or Supermix) was tested for its function using PCR at 1x strength. For the functional assay, a primer set was selected for its difficulty in its PCR in obtaining specific product with other Taq DNA polymerases. The sequences of the Rhod\_626 primer set primers are: forward primer (Rhod\_147F) 5'-AGG AGC TTA GGA GGG GGA GGT-3' (SEQ ID NO:4); reverse primer (Rhod\_773R) 5'-CAT TGA CAG GAC AGG AGA AGG GA-3' (SEQ ID NO:5).

Please replace paragraph [0203] with the following amended paragraph:

[0203] One more set of primers, in addition to the primer set above, was used for PCR functional assay of the reaction mixes for genomic and cDNA templates, respectively. For AccuPrime Taq Reaction Mix I and AccuPrime Taq SuperMix I, pUC19\_2.7 primers were used: forward primer: (pUC19\_2182F) 5'-TCA ACC AAT TCA TCC TGA GAA TAG T-3' (SEQ ID NO:6); reverse primer (pUC19\_2177R) 5'-TCA CCA GTC ACA GAA AAG CAT CTT AC-3' (SEQ ID NO:7). For AccPrime Taq Reaction Mix II and AccuPrime Taq SuperMix II, the Rhod\_626 primer set was used.

Please replace paragraph [0206] with the following amended paragraph (please note that paragraph title has not been added, but was underlined in the original text):

[0206]        TOPO TA cloning. Two separate amplicons from pUC19 were selected for their general usage and GC-richness. The first amplicon (multi-cloning site) was selected for frequency of its use. The commercial M13/pUC Amplification primers were used for the PCR reaction: forward primer (LTI, 18431-015) 5'-CCG AGT CAC GAC GTT GTA AAA CG-3' (SEQ ID NO:8); reverse primer (LTI, 18432-013) 5'-AGC GGA TAA CAA TTT CAC ACA GG-3' (SEQ ID NO:9). The second amplicon was selected for its GC-richness (62% GC content): forward primer (pUC19\_606f) 5'-CCA GTC GGG AAA CCT GTC GT-3' (SEQ ID NO:10); reverse primer (pUC19\_745r): 5'-ACC GCC TTT GAG TGA GCT GA-3' (SEQ ID NO:11). The amplicons were 136 and 159 bp long, respectively.

Please replace current pages 86-87 with amended pages 86-87 that are appended hereto.

Please replace current pages 90-93 with amended pages 90-93 that are appended hereto.

Please replace paragraph [0294] with the following amended paragraph (please note that paragraph title has not been added, but was underlined in the original text):

[0294]        Exo-nuclease activity. 100 pmol of oligonucleotide (36mer; 5'-GGG AGA CGG GGA ATT CGT CGA CGC GTC AGG ACT CTA-3' (SEQ ID NO:1)) was labeled with <sup>32</sup>P at the 5' end using 10 units of T4 polynucleotide kinase and 10 µCi of [γ-<sup>32</sup>P] ATP in 50 µl of 1x PNK exchange buffer. The reaction mix was incubated at 37°C for 30 min and the reaction was terminated by incubating the mix at 70°C for 10 min.

Unincorporated nucleotides were removed by eluting the reaction mix through Amersham-Pharmacia Micro Spin G-25 column twice following the manufacturers instruction.

Please replace paragraph [0296] with the following amended paragraph (please note that paragraph title has not been added, but was underlined in the original text):

[0296]        Host DNA contamination. Host DNA contamination assay was done by PCR using a primer set targeting a single copy gene in *E. coli* genome (*priA*) in the presence of denatured AccuPrime Protein II at 1x (300 ng per 50 µl reaction) or 2x (600 ng) concentration without added DNA template. Denaturation of AccuPrime Protein II was accomplished by treating the protein solution (100 µl at 0.52 mg/ml) with 50 µg of proteinase K digestion at 55°C for 1 hr. The peptidyl residues and the proteases were removed by extracting with phenol:chloroform:isoamyl alcohol (25:24:1) mix, followed by G-25 spin column (Pharmacia). The protein solution was treated as if it still contains the protein at the initial concentration for this purpose. Control reactions contain a known amount of *E. coli* genomic DNA in the absence of the protein as concentration markers in otherwise identical reactions. The *E. coli priA* 260 bp primer set was used: forward primer (*priA\_260\_F*) 5'-ACG CGC CGA TGT GGT ACT GGT TT-3' (SEQ ID NO:74); reverse primer (*priA\_260\_R*) 5'-GCG GTG GCC TGT TCG GTA TTC AA-3' (SEQ ID NO:75).

Please replace paragraph [0298] with the following amended paragraph:

[0298]        Functional PCR assay was performed to establish functionality of the purified AccuPrime Protein II. The assay was done using p53 2380 primer set with 100 ng of human genomic DNA (K562, genotyping grade) in 50 µl reactions except the increasing amount of AccuPrime Protein II from 100 to 600 ng per reaction at the increment of 100 ng, in the presence of 100 ng of AccuPrime Protein I. The Human p53 2380 bp primer set was used: forward primer (*p53\_2380\_F*) 5'-CCC CTC CTG GCC CCT GTC AT-3' (SEQ ID NO:76); reverse primer (*p53\_2380\_R*) 5'-GCA GCT CGT GGT GAG GCT CCC-3' (SEQ ID NO:77).



Please replace paragraph [0303] with the following amended paragraph:

[0303] 10x AccuPrime Pfx reaction mix was tested at 37 and 45°C for 7 and 4 days, respectively, which were equivalent to 1 yr of storage at -20°C. After the period of incubation, the reaction mix (or Supermix) was tested for its function using PCR at 1x strength. For the functional assay, a primer set was selected for its difficulty in its PCR in obtaining specific product with other Pfx DNA polymerases. The Human  $\beta$  globin (Hbg) 3.6 kb primer set was used: forward primer (Hbg\_3.6\_F) 5'-TTC CTG AGA GCC GAA CTG TAG TGA-3' (SEQ ID NO:78); reverse primer (Hbg\_3.6\_R) 5'-TAA GAC ATG TAT TTG CAT GGA AAA CAA CTC-3' (SEQ ID NO:79).

Please replace paragraph [0311] with the following amended paragraph:

[0311] Performance of AccuPrime Pfx DNA polymerase was compared with competitive high-fidelity PCR enzymes, such as Pfu Turbo DNA Polymerase (Stratagene, Cat. No. 600252, lot 1210608), Pwo DNA Polymerase (Roche, Cat. No. 1644 955, lot 49215324), Tgo DNA Polymerase (Roche, Cat. No. 3186 199, lot 90520522), and KOD Hot Start DNA Polymerase (Novagen, Cat. No. 71086-3, lot N33243). Each enzyme was used to amplify targets ranging from 822 bp to 6816 bp using 100 to 200 ng of human genomic DNA (K562, genotyping grade). Primers and their sequences are as follows: (#1, c-myc 822 bp primer set) forward primer (cmyc\_822\_F) 5'-CGG TCC ACA ACG AGC TCT CCA CTT G-3' (SEQ ID NO:20), reverse primer (cmyc\_822\_R) 5'-CTG TTT GAC AAA CCG CAT CCT TG-3' (SEQ ID NO:21); (#2, p53 2380 bp primer set) forward primer (p53\_2380\_F) 5'-CCC CTC CTG GCC CCT GTC AT-3' (SEQ ID NO:76), reverse primer (p53\_2380\_R) 5'-GCA GCT CGT GGT GAG GCT CCC-3' (SEQ ID NO:77); (# 3, Human  $\beta$  globin (Hbg) 3.6 kb primer set) forward primer (Hbg\_3.6\_F) 5'-TTC CTG AGA GCC GAA CTG TAG TGA-3' (SEQ ID NO:78), reverse primer (Hbg\_3.6\_R) 5'-TAA GAC ATG TAT TTG CAT GGA AAA CAA CTC-3' (SEQ ID NO:79); (#4, Rhod 6173 bp primer set) forward primer (Rhod\_575\_F) 5'- CCC TCT ACA CCT CTC TGC ATG GA -3' (SEQ ID NO:80), reverse primer (Rhod\_6748\_R) 5'- AGC AAC AAA ACC CAC CAC CGT

TA -3' (SEQ ID NO:81); (#5, Rhod 6816 bp primer set) forward primer (Rhod\_532\_F) 5'- GCC GTG GCT GAC CTC TTC ATG GT -3' (SEQ ID NO:82), reverse primer (Rhod\_6748\_R) 5'- AGC AAC AAA ACC CAC CAC CGT TA -3' (SEQ ID NO:81).

Please replace paragraph [0363] with the following amended paragraph:

[0363] ThermalAce™ DNA polymerase (Invitrogen Corp.) is a thermostable archaeobacterial enzyme having high processivity and 3' to 5' exonuclease proofreading activity (see US Patent No. 5,972,650). PCR was performed using ThermalAce™ DNA polymerase in conjunction with *M. jannachii* SSB (MjaSSB), *M. thermoautotrophicum* SSB (Mth SSB), and *S. solfataricus* SSB (SsoSSB). PCR reactions included 1-100 ng DNA template (K562 human genomic DNA, genotyping grade), 100 ng of each amplification primer (Rhod\_147F: 5'-AGG AGC TTA GGA GGG GGA GGT-3' (SEQ ID NO:4)) and Rhod\_773R: 5'-CAT TGA CAG GAC AGG AGA AGG GA-3' (SEQ ID NO:5)), 200 µM of each dNTP, ThermalAce™ buffer (Invitrogen Corp.), sterile water, and 2 units ThermalAce™ (add last). When present, SSB was included at concentrations of 0.1, 0.2 or 0.4 µg. Reactions were mixed thoroughly after adding ThermalAce™ and place on ice prior to thermocycling. Thermocycling parameters were as follows:

Please replace paragraph [0367] with the following amended paragraph:

[0367] We tested whether *Methanococcus jannachii* SSB (MjaSSB) can improve cycle sequencing with fluorescent dye terminators. Cycle sequencing was done using an ABI Prism® 377 DNA Sequencer, ABI Prism® BigDye™ Terminator Cycle Sequencing Kits, 0.25x BigDye ReadReaction Mix, and varying amounts of MjaSSB. Sequencing reactions included 500 ng of template (a plasmid having a gene cloned between attB sites) and 3.2 pmol of T7 promoter sequencing primer (5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO:83)) per 20 µl reaction. MjaSSB was included at 50 or 100 ng per reaction. Panel A in Figure 41 shows the result of a cycle sequencing reaction in the absence of SSB. The peak pileup (signal conflation) around position 35 and unreadable sequence thereafter may be caused by attB secondary

structure. Addition of MjaSSB obviated the peak pileup and increased the length of readable sequence (Panels B and C in Fig. 41).

Please replace current page 141 with amended page 141 that is appended hereto.

Please replace current pages 143-146 with amended pages 143-146 that are appended hereto.

Please replace current pages 149-152 with amended pages 149-152 that are appended hereto.

In the specification, after the Abstract of the Disclosure at page 156 and before the drawings, please insert the Sequence Listing (pages 1-46) that is appended hereto.